ZOOLOGICAL SCIENCE 7: 201–208 (1990)

© 1990 Zoological Society of Japan

Ultrastructural Studies of the Carotid Labyrinth in the Newt *Cynops pyrrhogaster*

TATSUMI KUSAKABE

Department of Anatomy, Yokohama City University School of Medicine, Yokohama 236, Japan

ABSTRACT—Fluorescence, light, and electron microscopic observations of the carotid labyrinth of the newt *Cynops pyrrhogaster* showed the presence of glomus cells in the intervascular stroma of the labyrinth. These cells contain many dense-cored vesicles (60 nm–100 nm in diameter) and their ultrastructure was similar to that of glomus cells reported in many other animal species. In some glomus cells, there were intranuclear inclusion bodies (0.1–0.3 μm in diameter), and long processes were in close contact with endothelial cells or with pericytes, which have not been reported in the carotid labyrinth so far. Two types of synapses (efferent and afferent) were found on the glomus cell surfaces. So-called reciprocal synapses were also found. On the basis of these findings, I conclude that the newt glomus cells may have a secretory as well as a chemoreceptor function.

INTRODUCTION

Amphibian carotid labyrinths have been morphologically studied by many workers (see Adams)[1]. Ishii *et al.* [2] confirmed in physiological experiments that they have an arterial chemoreceptor function analogous to that of the mammalian carotid body. The anuran carotid labyrinth contains glomus cells similar to those in mammalian carotid bodies and is innervated by glossopharyngeal and sympathetic nerves [3, 4]. It has been thought that the glomus cell functions as an element of the arterial chemoreceptor. In electron microscopy of the *Xenopus* carotid labyrinth, close contact of glomus cells with smooth muscle cells was reported, suggesting that the labyrinth might have another function other than chemoreception [5]. Most of the ultrastructural and physiological studies of the carotid labyrinth have been performed on anurans, and there are only a few morphological reports on the carotid labyrinth in urodelans [6, 7]. In the present study, some ultrastructural characteristics of the newt glomus cell which are different from those previously described in other amphibia are reported.

MATERIALS AND METHODS

Fifteen Japanese newts, *Cynops pyrrhogaster*, of both sexes weighing 8–10 g were used. The animals were anesthetized with urethane (5 mg/g body weight), and the region of the carotid labyrinth was exposed on both sides. Through a thin nylon tube inserted into the aortic trunk, the labyrinth was washed with Ringer solution and perfused with fixatives, then removed from the body. For fluorescence microscopy, specimens were immersed in Grillo's fixative [8] at 4°C for 20 hr. They were sectioned transversely at 60 μm. Observations were made with a fluorescence microscope (Olympus BHF) equipped with a HBO 200 high pressure mercury lamp, UG-5 and BG excitor filters, and a Y-475 barrier filter. For electron microscopy, the labyrinth was immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) at 4°C for 3 hr. After being washed in buffer solution, the specimens were divided into small blocks and postfixed in 1% osmium tetroxide buffered with 0.1 M cacodylate for 1 hr. After dehydration in a graded ethanol series, the specimens were embedded in Epon-alardite mixture. Sections of 1 μm were stained with toluidine blue

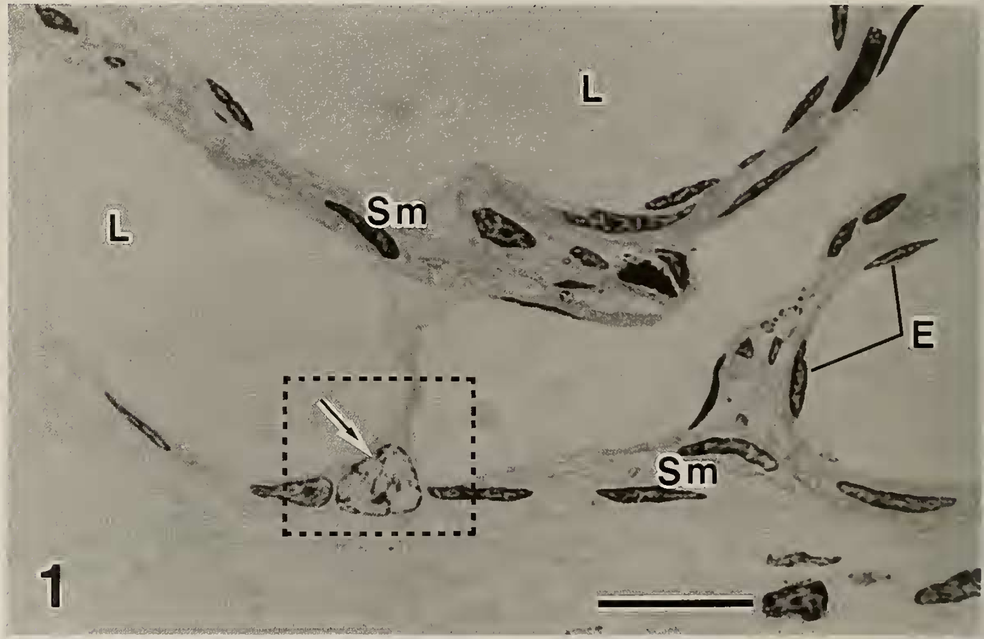
Accepted July 2, 1989
Received March 31, 1988

for light microscopy. Ultrathin sections were cut serially and double-stained with saturated uranyl acetate and Reynold's lead solution. electron micrographs were taken with a JEM 200-CX electron microscope at 80 kv.

RESULTS

Light and fluorescence microscopy

The carotid labyrinth had a maze-like structure consisting of the sinusoidal plexus and intervascular stroma. The stroma contained 6 main types of cells: glomus cells, smooth muscle cells, pericytes, fibroblasts, mast cells, and endothelial cells. The



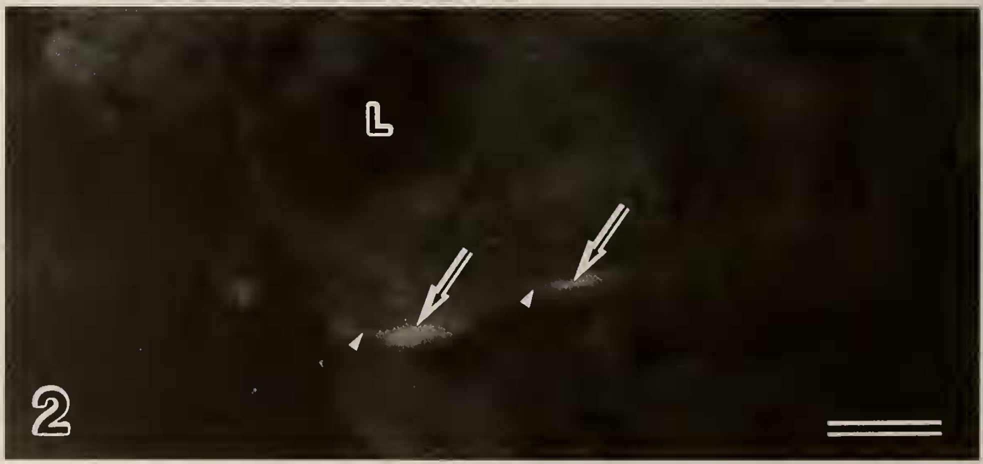


FIG. 1. Light micrograph of a toluidine blue-stained section of the carotid labyrinth. A glomus cell (arrow) is located near the lumen of a sinusoid (L). E, endothelial cells; Sm, smooth muscle cells. Scale bar, 20 μm.

FIG. 2. Fluorescence micrograph of the carotid labyrinth. Two greenish-yellow fluorescent cells (arrows) with long processes (arrowheads). L, lumen of sinusoid. Scale bar, 50 μm.

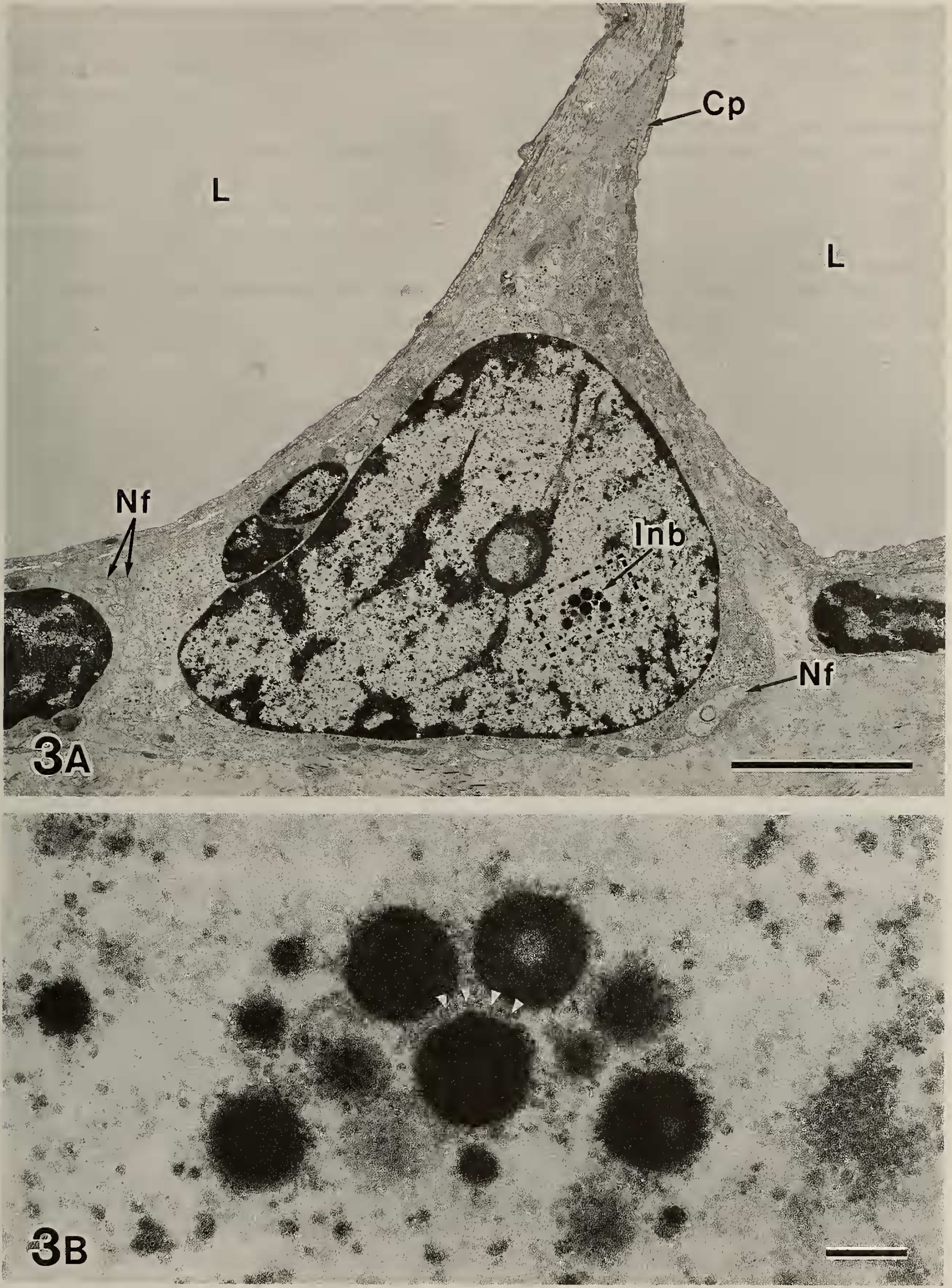


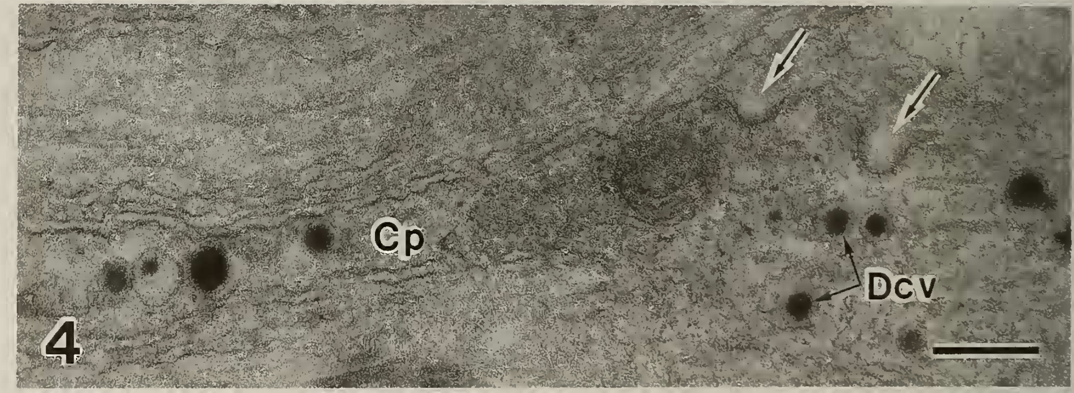
Fig. 3. A) Electron micrograph in low magnification of the glomus cell. The glomus cell (the boxed area in Figure 1) contains numerous cytoplasmic granules. Cp, cytoplasmic projection. Inb, intranuclear inclusion bodies; L, lumen of sinusoid; Nf, nerve fiber. Scale bar, 5 μm. B) High magnification of the intranuclear inclusion bodies. Between bodies small dense materials lie in a uniform array at regular intervals (arrowheads). Scale bar, 0.2 μm.

glomus cells had pale cytoplasm and a large oval nucleus, and were located singly or in clusters (50–60 μm in diameter) of 3–4 cells between connective tissues and smooth muscle cells (Fig. 1). In fluorescence microscopy, the glomus cells were identified as small, intensely fluorescent (greenish yellow) cells with long processes (Fig. 2).

Electron microscopy

The sinusoidal wall was composed of extremely thin endothelial cells. In the subendothelial stroma, several cellular elements and extracellular components such as collagenous fibers and amorphous ground substance were located. Enveloped by thin processes of supporting cells, the glomus cells were located in the vascular stroma singly or in clusters (Fig. 3A). Occasionally, a part of cell surface was directly in contact with the connective tissue, losing its covering of supporting cells. Between the clustered cells desmosome-like junctions were often observed. The cells were usually oval. A single oval nucleus contained dispersed clumps of heterochromatin. Near the nucleolus, a group of electron-dense inclusion bodies of various sizes (0.1–0.3 μm in dimeter) was sometimes observed (Fig. 3A). At high magnification, many small dense particles were attached to the surface of intranuclear inclusion bodies (Fig. 3B).

Numerous dense-cored vesicles (60–100 nm in diameter) were scattered throughout the cytoplasm. Coated pits were often found on the surface of the cell body and its processes (Fig. 4). A well-developed Golgi complex was found in the perinuclear area, and in its vesicles small newly synthesized low-density granules were seen. A relatively small amount of granulated endoplasmic



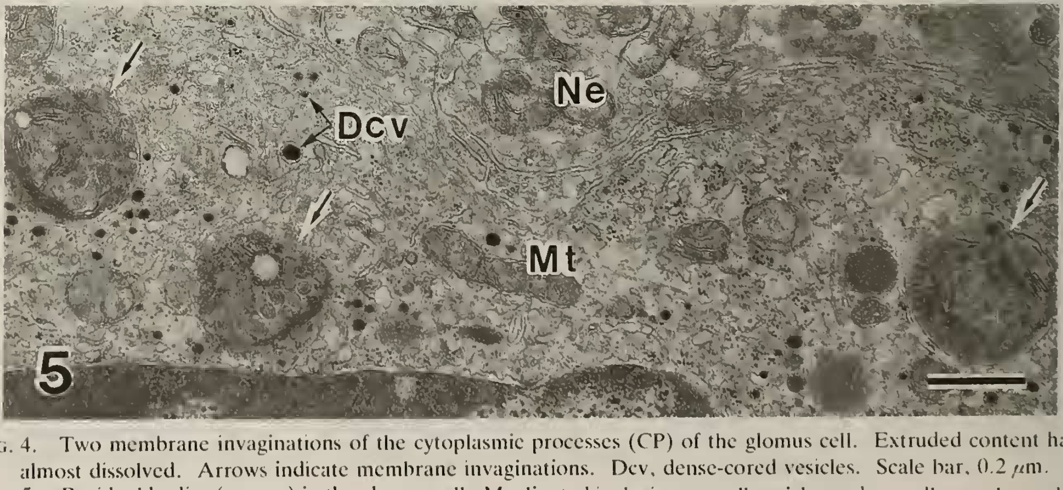


FIG. 4. Two membrane invaginations of the cytoplasmic processes (CP) of the glomus cell. Extruded content has almost dissolved. Arrows indicate membrane invaginations. Dcv, dense-cored vesicles. Scale bar, 0.2 μm.

FIG. 5. Residual bodies (arrows) in the glomus cell. Myelinated inclusions, small vesicles and a small vacuole can be seen. Dcv, dense-cored vesicles; Mt, mitochondria. Ne, nerve ending. Scale bar, 0.5 μm

reticulum, numerous free ribosomes and oval elongated mitrochondria were dispersed throughout the cytoplasm. Centrioles were located near the nucleus. Large residual bodies, a kind of lysosome, were also observed. They were round and about 0.7 μm in diameter with myelinated inclusions and small vesicles (Fig. 5). Losing their covering of supporting cells, long thin cytoplasmic projections of the glomus cells extended toward the endothelium. Some of them were longer than 20 μm and closely associated with the endothelial cells (g-e connection) (Fig. 6A) or with the pericytes (g-p connection) (Fig. 6B) without visible intervening substances.

Enclosed by supporting cells, nerve endings lay close to the glomus cells to make synapses. Two types of synapses were distinguished on the glomus cell. Most of the large synapses were morphologically efferent and were characterized by the accumulation of clear vesicles (40–60 nm in diameter). At the junction the membrane of the nerve ending was thicker than the cell membrane (Fig. 7A). Small afferent type synapses were sometimes observed; specialized membrane thickenings were conspicuous on the cell membrane where dense-cored vesicles were aggregated (Fig. 7A). So-

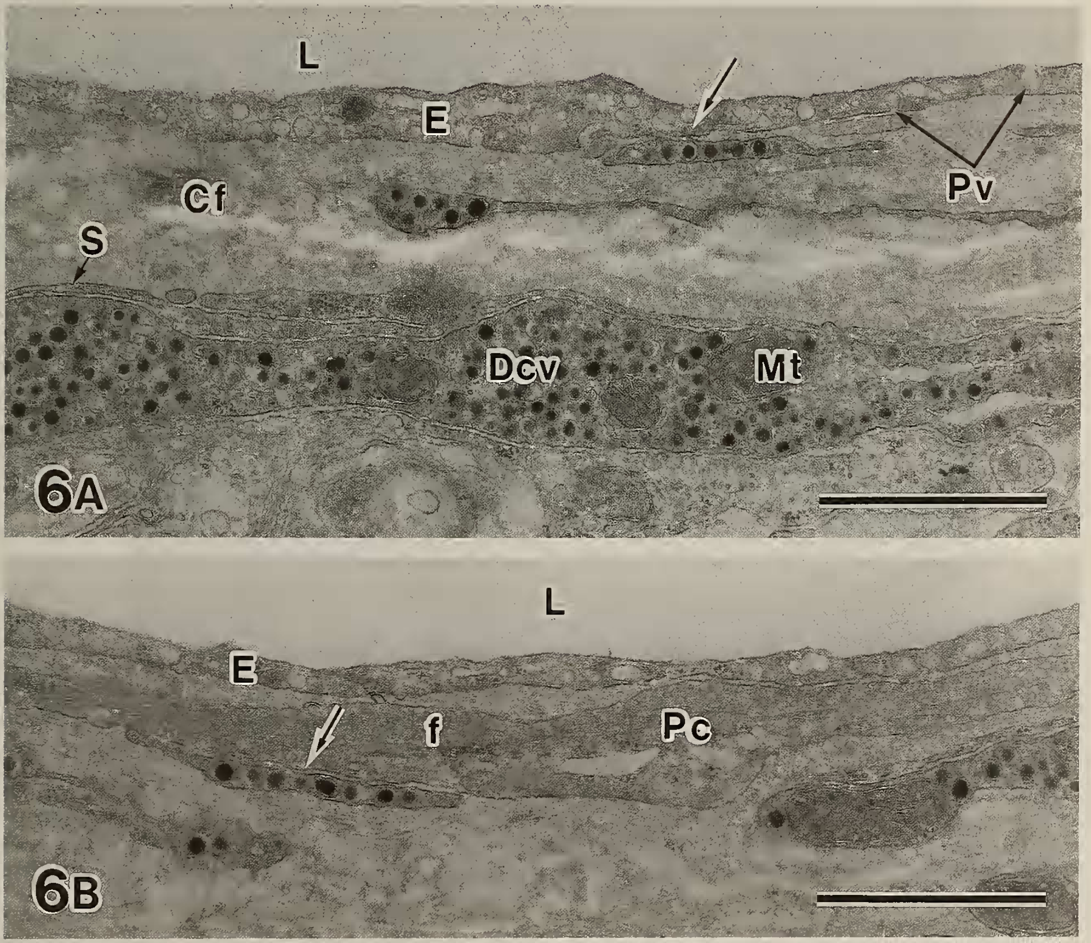


Fig. 6. A) Three cytoplasmic processes extend into the intervascular stroma. The inner two processes lose the covering of supporting cell cytoplasm (S), and luminal one makes g-e connection (arrow). Cf, collagenous fiber; Dcv, dense-cored vesicles; E, endothelial cell; L, lumen of sinusoid; Mt, mitochondria; Pv, plasmalemmal vesicles. Scale bar, 1 μm. B) A pericyte (Pc) directly connect with the process of a glomus cell (arrow). E, endothelial cell; f, filaments; L, lumen of sinusoid. Scale bar, 1 μm.

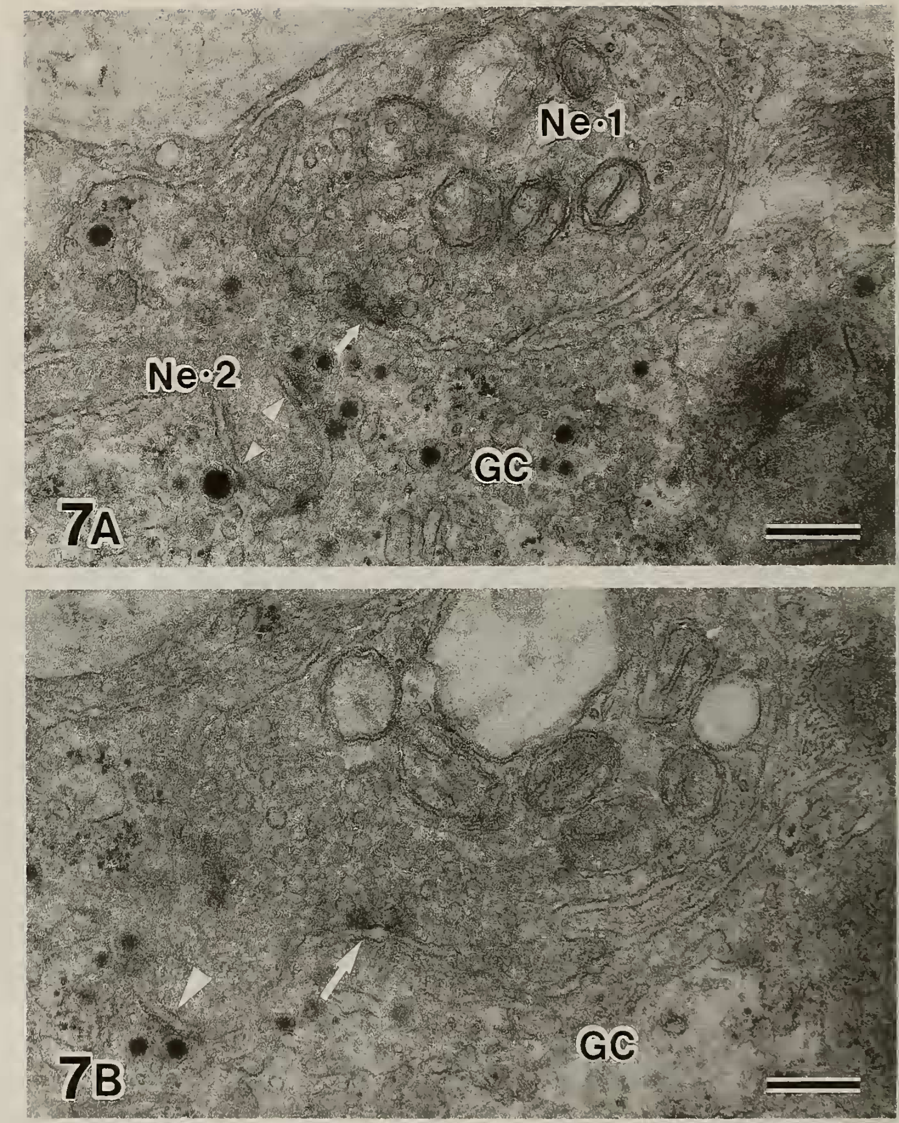


FIG. 7. A) Synaptic junctions between the glomus cell and nerve ending. The arrow and arrowheads indicate efferent and afferent type synapses, respectively. One nerve ending (Ne·1) which contains numerous clear vesicles and some mitochondria synapses with the glomus cell (Gc). The synapse shows asymmetrical membrane thickning on the Ne·1 side. Many synaptic vesicles accumulate at this junctional region (arrow). Another nerve ending (Ne·2) without clear veiscles synapses with the same glomus cell (Gc). Asymmetrical membrane thickenings are found on the glomus cell side. A few dense-cored vesicles aggregate at this synaptic region (arrowheads). Scale bar, 0.2 μm. B) So-called reciprocal synapses between the glomus cell and nerve ending. The arrow and arrowhead show efferent and afferent type synapses, respectively. A and B are semi serial sections. Scale bar, 0.2 μm.

called reciprocal synapses consisting of efferent and afferent type synapses on the same nerve ending were also observed (Fig. 7B).

DISCUSSION

The carotid labyrinth of the urodeles consists of a vascular maze [1, 9] and contains cells with many dense-cored vesicles [6]. The present electron microscopic study in the newt *Cynops pyrrhogaster* showed that these cells (glomus cells) are situated in the vascular stroma as isolated cells or in clusters of 3 or 4 cells, and their ultrastructure is similar to that of glomus cells observed in arterial chemoreceptor regions of anurans [4, 10]. In the toad *Bufo vulgaris,* the carotid labyrinth has been physiologically confirmed to have an arterial chemoreceptor function similar to that of the mammalian carotid body [2]. The glomus cell has long been known to be a chemoreceptor, but in recent years it has been postulated to have a secretory function as well [5, 11]. Although there is no direct evidence, the structural characteristics of the newt carotid labyrinth point to a chemoreceptor function, in which the glomus cells play the main role.

In the present paper, I have reported for the first time the presence of intranuclear inclusion bodies in the glomus cell nucleus. Intranuclear inclusion bodies have been observed only in clearly defined secretory cells: in the intestine [12] and in the colon [13] of the horse, in the epididymis of the dog [14], and in the adenohypohysis of the rabbit [15]. Although their origin and role are as yet unknown, they may be considered a characteristic feature of the secretory function. This finding may indicate a secretory function of the glomus cell as stated above, or it may show that intranuclear inclusion bodies exist in more extensive cell domains than so far accepted.

In addition, the g-e connection was observed for the first time in amphibian glomus cells. Kondo [16] and Ookawara *et al.* [17] observed close contact of granule-containing cells with endothelial cells in the domestic fowl, and Kondo [16] proposed a secretory function for the granule-containing cells there. Since the vesicles in the endothelial cells are commonly accepted to involved in intracellular transport [18, 19], catecholamines contained in dense-cored vesicles might be released into the blood vessels. However these might also be structures facilitating blood gas uptake by glomus cells for chemoreception. On the other hand, the g-p connection suggests that the vascular tone might be modified by catecholamine in glomus cell granules, if catecholamine releasing occurs in this region.

Two types of synapses, efferent and afferent, were reported in toad carotid labyrinth [4] and in tortoise carotid artery [20]. Also in this study, afferent and efferent synapses were observed between the nerve endings and glomus cells as described by Kobayashi [6]. Furthermore Yamauchi [21] reported reciprocal synapses in the toad carotid labyrinth. Serial sections suggested that all nerve endings may be reciprocal in the newt carotid labyrinth.

In conclusion, the newt glomus cells may have a secretory function in addition to their chemoreceptor function.

ACKNOWLEDGMENTS

I wish to thank Prof. M. Asashima of the Department of Biology for kindly supplying animals, Dr. R. C. Goris of Department of Anatomy, Yokohama City University for his help in preparing the manuscript and Drs. Kosei Ishii and Kazuko Ishii for their critical reading of the manuscript. This work was supported by Grants-in-Aid (No. 63770024) from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1 Adams, W. E. (1958) The comparative morphology of the carotid body and carotid sinus. Charles C. Thomas, Springfield, pp. 202–214.

2 Ishii, K., Honda, K. and Ishii, K. (1966) The function of the carotid labyrinth in the toad. Tohoku J. exp. Med., **88**: 103–116.

3 Rogers, D. C. (1963) Distinct cell types in the carotid labyrinth. Nature, **200**: 492–493.

4 Ishii, K. and Oosaki, T. (1969) Fine structure of the chemoreceptor cell in the amphibian carotid labyrinth. J. Anat. (Lond), **104**: 263–280.

5 Ishii, K. and Kusakabe, T. (1982) The glomus cell of the carotid labyrinth of *Xenopus laevis.* Cell Tissue Res., **224**: 459–463.

6 Kobayashi, S. (1971) Comparative cytological studies of the carotid body. 2. Ultrastructure of the synapse on the chief cell. Arch. histol. jap., **33**: 397–420.

7 Wislang, M. R. (1965) The carotid labyrinth in two species of urodeles. J. Anat. (Lond), **99**: 949.

8 Grillo, M. A., Jacobs, L. and Comroe, Jr. J. H. (1974) A combined fluorescence histochemical and electron microscopic method for studying special monoaminecontaining cells (SIF cells). J. Comp. Neurol., **153**: 1–14.

9 Ishida, S. (1954) So-called carotic body of the

amphibia. Igaku Kenkyu (Fukuoka), **24**: 1024–1050.

10 Ishii, K. Ishii, K. and Kusakabe, T. (1985) Chemo- and baroreceptor innervation of the aortic trunk of the toad *Bufo vulgaris*. Respir. Physiol., **60**: 365–375.

11 Kusakabe, T., Ishii, K. and Ishii, K. (1987) A possible role of the glomus cell in controlling vascular tone of the carotid labyrinth of *Xenopus laevis*. Tohoku J. exp. Med., **151**: 395–408.

12 Dual, D. E. (1980) The origin of nuclear bodies: A study of the undifferentiated epithelial cells of the equine small intestine. Am. J. Anat., **157**: 61–70.

13 Pfeiffer, C. J., Marry, M. J. and Legends, L. (1987) The equine colonic mucosal granular cell: Identification and X-ray microanalysis of apical granules and nuclear bodies. Anat. Rec., **219**: 258–267.

14 Nicander, L. (1964) Fine structure and cytochemistry of nuclear inclusions in the dog epididymis. Exp. Cell Res., **34**: 533–541.

15 Foster, C. L., Young, B. A., Allanson, M. and Cameron, E. (1965) Nuclear inclusions in the adenohypophysis of the rabbit. J. Endocrinol., **33**: 159–160.

16 Kondo, H. (1974) On the granule-containing cells in the aortic wall of the young chick. Anat. Rec., **178**: 253–266.

17 Ookawara, S., Suzuki K., Yoshida, Y. and Ooneda, G. (1974) Monoamine-storing cells in the media of the thoracic aorta of *Gullus domesticus*. Cell Tiss. Res., **151**: 309–316.

18 Palade, G. E. (1953) Fine structure of blood capillaries. J. appl. Physics, **24**: 1424.

19 Simionescu, N., Simionescu, M. and Palade, G. E. (1975) Permeability of muscle capillaries to small heme-peptides. Evidence for the existence of patent transendothelial channels. J. Cell Biol., **64**: 586–607.

20 Kusakabe, T., Ishii, K. and Ishii, K. (1988) Dense granule-containing cells in the arterial chemoreceptor area of the tortoise (*Testudo hermanni*). J. Morphol., **197**: 183–191.

21 Yamauchi, A. (1977) On the recepto-endocrine property of granule-containing (GC) cells in the autonomic nervous system. Arch. histol. jap., **40**, Suppl: 147–161.